SPECIFICATION

ORGANIC ANION TRANSPORTER AND GENE CODING FOR THE SAME

TECHNICAL FIELD

The present invention is related to the genes and their encoding polypeptides, which are related to the transport of organic anions.

BACKGROUND ART

The kidney plays important roles in the excretion of endogenous compounds and xenobiotics. Anionic substances including drugs are excreted via carrier-mediated pathway(s) into the urine. The first step of this secretion is the uptake of organic anion from the peritubular plasma across the basolateral membrane of the proximal tubule cells.

The basolateral uptake of the organic anions has been studied using several techniques, such as perfusion of excised kidney, or membrane vesicles of isolated tubule cells. In these studies, para-aminohippurate (PAH) has been widely used as a test substrate. During these studies, it has been supposed that the organic anion transporter responsible for the basolateral uptake of organic anions was an organic anion/dicarboxylate exchanger.

There are, however, limitations in the previous techniques for precise analysis of the organic anions transport, such as the networks of transport between different transporters and the drug-drug interaction against a single molecule. Thus, the isolation of the organic anion transporter molecule which enables more precise analysis of the organic anion transporter has been eagerly awaited .

So far, several transporter molecules which are expressed in the liver have been isolated (Hagenbuch, B. et al. Proc. Natl. Acad. Sci. U.S.A. 88, 10629-10633, 1991, Jacquemin, E. et al. Proc. Natl. Acad. Sci. U.S.A. 91, 133-137, 1994). The cDNA cloning of organic cation transporter (OCT1), which is expressed in the kidney and the liver, was also reported (Grundemann, D. et al. Nature 372, 549-52, 1994).

As a sodium-dependent dicarboxylate cotransporter, the cDNA encoding sodium-dicarboxylate co-transporter (NaDC-1) was reported (Pajor, A.M. J. Biol. Chem. 270, 5779-5785, 1995)

Recently, OAT-K1, an isoform of oatp was isolated (Saito, H. et al. J. Biol. Chem. 271, 20719-20725, 1996). Oatp is organic anion transporting polypeptide which is expressed in the liver and mediates the sodium-independent transport of organic anions. OAT-K1 is expressed in the renal proximal tubules, however, the transport properties of OAT-K1 was distinct from that of the organic anion/dicarboxylate exchanger of the renal proximal tubule cells.

DISCLOSURE OF THE INVENTION

The aim of the present invention is to provide novel genes and the gene products, which are related to the renal transport of organic anions. The other aims of this invention will be explained in the following.

BRIEF EXPLANATIONS OF THE FIGURES

FIG. 1 shows the uptake of glutarate by the oocytes injected with rat sodium dependent dicarboxylate cotransporter (rNaDC-1) cRNA.

FIG. 2 shows the uptake experiment using the oocytes injected with rat kidney mRNA and/or rNaDC-1 cRNA.

FIG. 3 shows Hydropathy analysis of rat organic anion transporter OAT1.

FIG. 4 shows Northern blot analysis of rat organic anion transporter OAT1 using mRNAs derived form various rat tissues.

FIG. 5 shows the effect of pre-incubation with glutarate, or co-expression with rNaDC-1 was examined in oocytes injected with rat OAT1

FIG. 6 shows the effect of extracellular sodium ion on the rat OAT1-mediated uptake of PAH in oocytes injected with OAT1 cRNA.

FIG. 7 shows transport rate of different concentrations of PAH in oocytes injected with rat OAT1 cRNA was examined.

FIG. 8 shows *Cis*-inhibitory effect of various anionic substances on the rat OAT1-mediated uptake of PAH was examined.

FIG. 9 shows the result of that radio labeled drugs was examined whether they were transported by rat OAT1.

BEST MODE FOR CARRYIING OUT THE IVENTION

We isolated a novel cDNA which encodes a membrane protein, OAT1, from the rat kidney. We also isolated the human homolog of OAT1. We expressed rat and human OAT1 in the *Xenopus laevis* oocytes, and successfully demonstrated that these proteins mediated the transport of organic anions. Thus we could complete this invention.

The proteins whose amino acid sequences are described in A, B, C and D are all included in this invention.

(A) The protein whose amino acid sequence is shown in SEQUENCE No. 1.

(B) Proteins whose amino acid sequences are identical to that shown in SEQUENCE No. 1 except that several amino acid residues are deleted, substituted or added in it.

Despite of these changes, the protein must possess the ability to transport organic anions.

(C) The protein whose amino acid sequence is shown in SEQUENCE No. 2.

(D) Proteins whose amino acid sequences are identical to that shown in SEQUENCE No. 2 except that several amino acid residues are deleted, substituted or added in it. Despite of these changes, the protein must possess the ability to transport organic anions.

The DNAs whose nucleotide sequences are described in a, b, c and d are also includes in this invention.

(a) The DNA whose nucleotide sequence is shown in SEQUENCE No. 1.

(b) DNAs which can hybridize the DNA shown in SEQUENCE No. 1 in stringent condition, and encode the proteins possessing the ability to transport organic anions.

(c) The DNA whose nucleotide sequence is shown in the SEQUENCE No. 2.

(d) DNAs which can hybridize the DNA shown in SEQUENCE No. 2 in stringent condition, and encode the proteins possessing the ability to transport organic anions.

The novel protein of the present invention (OAT1: organic anion transporter 1) which possesses the ability to transport organic anions, is expressed predominantly in the renal proximal tubule cells.

The transport rate of organic anions via OAT1, i.e. the uptake rate of organic anions into the cell expressing OAT1, is stimulated by dicarboxylates present in the cells. This fact indicates that OAT1 is an organic anion/dicarboxylate exchanger. The dicarboxylates which are effluxed in exchange for organic anion via OAT1, are taken up by the sodium-dicarboxylate cotransporter from the extracellular fluid. Thus, dicarboxylate are recycled for the OAT1-mediated transport of organic anions.

The novel protein of the present invention, OAT1, possesses the ability to

transport (take up) various organic anions, such as cycic nucleotides, prostaglandins, urate, antibiotics, diuretics and anticancer drugs. Since chemical structures of these substances are diverse, the substrate selectivity of OAT1 is considered to be very wide.

The amino acid sequence of OAT1 shows no similarity to that of the previously isolated renal organic anions transporter OAT-K1. Thus, OAT1 belongs to distinct transporter family.

Sub C9 The SEQUENCE NO. 1 shown in the table depicts the total nucleotide sequence of rat OAT1 cDNA (approximately 2.2 kb) with the deduced amino acid sequence (551 amino acid residue) encoded by the open reading frame of rat OAT1 cDNA.

Sub C10 The SEQUENCE NO. 2 shown in the table depicts the total nucleotide sequence of human OAT1 cDNA (approximately 2.2 kb) with the deduced amino acid sequence (563 amino acid residue) encoded by the open reading frame of human cDNA.

We searched for the DNA database (GeneBank and EMBL) and protein database (NBRF and SWISS-PROT) for the homologues sequence of OAT1. We could not find any homologues sequences of OAT1 in the sequences whose function had been clarified.

Sub C11 In addition to the amino acid sequence shown in SEQUENCE NO. 1 and NO. 2, the present invention includes the following proteins. Proteins whose amino acid sequences are identical to that shown in SEQUENCE NO. 1 except that several amino acid residues are deleted, substituted or added in it. The extent of changes in amino acid sequence of these proteins are acceptable when the product proteins possess the ability to transport organic anions. Usually, numbers of the changed amino acid residues are

between one to 110, preferably 1 to 55. These amino acid sequences show 80 %, preferably 90 %, identity to that shown in SEQUENCE NO: 1 or NO: 2.

Sub C12 In addition to the DNAs with the nucleotide sequences shown in SEQUENCE NO. 1 and NO. 2, the present invention includes DNAs which can hybridize the cDNA shown in SEQUENCE NO. 1 and No. 2. The proteins encode by these DNAs must possess the ability to transport organic anions. Usually, these DNAs show more than 70 %, preferably 80 %, identity to those shown in SEQUENCE NO. 1 or NO. 2. These DNAs include mutated genes found in nature, artificially ? mutated genes and the genes derived from other species of living cells.

The stringent condition in hybridization screening, which we refer to in this invention, indicates that hybridization is performed at 37-42 °C for approximately 12 hours in 5 X SSC (Standard Saline Citrate) solution, or in the hybridization solution with equivalent concentrations of salts, followed by washing in 1 X SSC solution. If more high stringency condition is required, washing process can be performed in 0.1 X SSC or solutions with equivalent concentrations of salts.

The homologues genes encoding the organic anion transporter of the present invention, can be obtained from other species, such as the dogs, bovines, horses, gouts, sheep, monkeys, pigs, rabbits and mouse, using homology screening. For this purpose, cDNA library can be constructed from the kidney or culture cells of the aimed species of animals.

In addition to the homology screening, the isolation of the genes can be performed using expression cloning technique.

In the following, we will explain the method of expression cloning briefly, which we used for the isolation of the renal organic anion transporter.

mRNA (poly $(A)^+$ RNA) obtained from the rat kidney is divided into fractions

according to their size, and each fraction of mRNA is injected into Xenopus laevis oocytes with cRNA of rat sodium-dependent dicarboxylate cotransporter.

The cDNA sequence of rabbit sodium dicarboxylate cotransporter (NaDC-1) was already reported (Pajor, A.M. J. Biol. Chem. 270, 5779-5785, 1995), therefore, the cDNA of rat sodium dicarboxylate cotransporter (NaDC-1) can be easily isolated. The complementary RNA (cRNA) for rNaDC-1 cDNA can be synthesized *in vitro* using RNA polymerases, such as T3 or T7 RNA polymerase.

Oocytes injected with rat kidney mRNA and the cRNA of rNaDC-1 are examined for the uptake rate of radio-labeled organic anions, such as PAH, and the mRNA fractions showing the highest transport rate of PAH can be determined. The cDNA library can be constructed from these selected fractions, which should contain concentrated mRNA for the PAH transporter. cRNAs can be synthesizes from the constructed cDNAs and injected into oocytes with the rNaDC-1 cRNA. By repeating the screening, the cDNA which encodes the PAH transporter can be isolated.

The sequence of the obtained clone can be determined by dideoxytermination method, and the deduced amino acid sequence encoded can be predicted.

Whether the cDNA obtained really encodes the organic anion transporter can be verified as follows. cRNA synthesized from the isolated cDNA clone is injected into Xenopus oocytes, and ability of the expressed protein to transport of organic anions can be examined as described elsewhere (Kanai, Y. and Hediger, M.A. (1992) Nature 360, 467-471).

Functional analysis of the organic anion transporter, such as the exchange property of OAT1, can be examined using the oocytes expressing OAT1.

Using the cDNA of rat OAT1, homologues DNAs or chromosomal genes

derived from different tissues or different animals can be obtained from appropriate cDNA or genomic library.

Based on the sequence of this invention shown in SEQUENCE NO. 1 and NO. 2, sets of PCR (polymerase chain reaction) primers can be designed by which cDNA probes can be synthesized to search the cDNA or genomic library.

cDNA library or genomic DNA library can be constructed using methods described , for example, in "Molecular Cloning" edited by Sambrook, J., Fritsch, E.F., and Maniatis, T. Cold Spring Harbor Laboratory Press, 1989. Commercially available library can also be used.

The organic anion transporter of this invention can be produced by the molecular recombination technique. For example, the cDNA encoding the organic anion transporter is subcloned into expression vectors, followed by transformation p f appropriate host cells with them. For expression systems to produce polypeptides, host cells, such as bacteria, yeast, insect and mammalian cells can be used . Among these, insects cells and mammalian cells are preferable to obtain the proteins with functions.

When the organic anion transporter is required to be expressed in the mammalian cells, the cDNA encoding the organic anion transporter should be subcloned into mammalian expression vectors, such as retrovirus vectors, papilloma virus vectors, vaccinia virus vectors and SV40 vectors. In this case, the cDNA of organic anion transporter must be inserted after? the promoter regions, such as SV40 promoter, LTR promoter and elongation 1α promoter. Then appropriate animal cells are transformed with the recombinant vectors containing the organic anion transporter cDNA. The mammalian cells, such as COS7 cells, CHO cells, Hela cells, primary culture cells derived from the kidney, LLC-PK1 cells and OK cells, can be used for this purpose.

The cDNAs which can be used for the above mentioned purpose are not restricted to those shown in SEQUENCE NO. 1 and NO. 2. Since each amino acid is encoded by several types of codon, cDNAs which encode the proteins with the amino acid sequences shown in SEQUENCE NO. 1 and NO. 2 can be designed based on information of codons. Any codons, which encode the desired amino acid, can be selected, and cDNAs inducing more efficient expression may be designed considering the codon preference in the host cells. The designed cDNAs can be obtained by chemical DNA synthesis, digestion and ligation technique, and site-directed mutagenesis method. The methods of the site directed mutagenesis are described elsewhere (Mark, D.F., et al., Pro Nat Aca Sci, vol 81, 5662~5666, 1984)

The nucleotides which can hybridize the cDNA of OAT1 in high stringent condition can be used as probes to detect the organic anion transporters. In addition, they can be used to alter the expression level of the organic anion transporter, such as antisense-nucleotide, ribozyme and decoy. For this purpose, continuous nucleotides more than 14 base pairs, or their complementary nucleotide sequences can be used. If more specificity is required, more longer fragments, for example more than 20 to 30 nucleotides sequence, can be applied.

The antibody against the organic anion transporter of this invention can be obtained, using the fragments of the organic anion transporter or the synthesized polypeptides with the partial sequences which have equivalent immunochemical properties. Polyclonal antibody can be obtained by the ordinary immunizing method. i.e. immunize the rat or rabbit with antigen, and recover the serum. Monoclonal antibody can be obtained by the ordinary method such as hybridoma technique. These antibody can be used to detect or purify the organic anion transporter

In the following, we will explain the present invention precisely, however, this

invention is not restricted to the following description

This invention has been performed, if not indicated otherwise, using methods described in the "Molecular Cloning" (edited by Sambrook, J., Fritsch, E. F., and Maniatis, T., Cold Spring Harbor Laboratory Press, 1989), or using commercially available reagents and kits according to the manufacturer instructions, .

EXAMPLES

EXAMPLE 1: CLONING OF RAT ORGANIC ANION TRANSPORTER

(1) cDNA cloning of rat sodium-dicarboxylate co-transporter (rNaDC-1), and the preparation of rNaDC-1 cRNA

A non-directional cDNA library was prepared from rat kidney poly(A)$^+$ RNA using commercially available kit (Superscript Choice system, GIBCO BRL) and was ligated to λZipLox EcoRI arms (GIBCO BRL). A PCR product corresponding to nucleotides 1323 1763 of the rabbit sodium dicarboxylate transporter (NaDC-1) (Pajor, A.M. (1995) J. Biol. Chem. 270, 5779-5785) was labeled with ^{32}P-dCTP. A rat cDNA library was screened with this probe at low stringency. Hybridization was done overnight in the hybridization solution at 37°C and filters were washed finally at 37°C in 0.1X SSC / 0.1% SDS. The hybridization solution contains 5 X SSC, 3 X Denhardt's solution, 0.2% SDS, 10% dextran sulfate, 50 % formamide, 0.01% Antifoam B, 0.2 mg/ml denatured salmon sperm DNA, 2.5 mM sodium pyrophosphate and 25 mM MES, pH 6.5. cDNA inserts in positive λZipLox phage were recovered in plasmid pZL1 by *in vivo* excision and further subcloned into pBluescript II SK- (Stratagene) for sequencing and *in vitro* transcription.

rNaDC-1 cRNA was synthesized *in vitro* using the rNaDC-1 cDNA as a template.

Xenopus laevis oocyte expression studies and uptake measurements were performed as described elsewhere (Kanai, Y. and Hediger, M.A. (1992) Nature 360, 467-471, 1992). Defolliculated oocytes were injected with i*n vitro* transcribed cRNA of rNaDC-1, and ^{14}C-glutarate uptake was examined in ND96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, 5 mM HEPES, pH 7.4).

As shown in Fig. 1, the oocytes injected with rNaDC-1 cRNA showed the sodium-dependent uptake of glutarate, indicating that the isolated rNaDC-1 encodes the rat sodium-dependent dicarboxylate cotransporter.

(2) Cloning of the rat renal organic anion transporter OAT1.

The expression cloning of organic anion transporter 1 (OAT1) was performed using the method described elsewhere (Kanai, Y. and Hediger, M.A. (1992) Nature 360, 467-471, 1992)

Four hundreds μg of rat kidney poly(A)$^+$ RNA was size fractionated as described elsewhere (Kanai, Y. and Hediger, M.A. (1992) Nature 360, 467-471, 1992) using preparative gel electrophoresis (BIO RAD, Model 491 Prep cell).

Then we co-injected poly(A)$^+$ RNAs of each fraction together with rNaDC-1 cRNA into oocytes. Before uptake study, the oocytes were routinely preincubated for two hours in ND96 solution containing 1 mM glutarate for 2 hours.

Uptake experiment was performed in oocytes injected with poly(A)$^+$ RNAs of each fraction together with rNaDC-1 cRNA. ^{14}C-PAH (50 μM) uptake was measured in ND96 solution without glutarate for 1 hour. In this experiment, only those oocytes injected with both poly(A)$^+$ RNAs of each fraction and rNaDC-1 cRNA showed

significant uptake of PAH: in contrast oocytes injected with only poly(A)$^+$ RNAs of each fraction or rNaDC-1 cRNA did not show any uptake of PAH (Fig. 2).

We determined the cRNA fractions (1.8 - 2.4 kilobase (kb) poly (A)$^+$ RNA), which induced the highest PAH uptake rate when injected with rNaDC-1 cRNA into X. oocytes. Then a directional cDNA library was constructed from these fractions using Superscript Plasmid system (GIBCO BRL), and was ligated into the Sal I and Not I site of pSPORT 1. Recombinants were electroporated into Electro Max DH10B competent cells (GIBCO BRL). Approximately 500 colonies were grown on nitrocellulose membrane. Plasmid DNA was purified from colonies of each plate. Capped cRNA was synthesized in vitro after linearization of each plasmid DNA with Not I.

Then we co-injected cRNA synthesized from each filter together with 2 ng rNaDC-1 cRNA into oocytes. When ^{14}C-PAH uptake was detected on a particular group, it was subdivided into several groups, and further screened.

After screening of eight thousands clones, we isolated a single clone (OAT1), which mediated the significant uptake of PAH.

Deleted clones obtained by Kilo-Sequence Deletion kit (Takara, Japan) or specially synthesized oligonucleotide primers were used for sequencing of OAT1 cDNA. OAT1 were sequenced by dideoxytermination method using Sequenase ver. 2.0 (Amersham) or Dye Primer Cycle Sequencing Kit (Applied Biosystems).

Then we determined the nucleotide sequence of OAT1, and deduced the coding region of OAT1 cDNA and the amino acid sequence encoded.

The nucleotide SEQUENCE NO. 1 is the sequence of OAT1

Kyte-Doolittle hydropathy analysis (Kyte, J. and Doolittle, R.F. (1982) J. Mol. Biol . 157, 105-132) of OAT1 predicts twelve putative membrane-spanning domains (Fig. 3). Five N-glycosylation sites are predicted in the first hydrophilic loop. There are

4 putative protein kinase C-dependent phosphorylation sites in the hydrophilic loop between 6 th and 7 th transmembrane domains.

(3) The tissue distribution of OAT1 analyzed by Northern blot

The tissue distribution of OAT1 mRNA was examined. Three μg of poly $(A)^+$ RNA prepared from various rat tissues were electrophoresed on a 1% agarose/formaldehyde gel and transferred to a nitrocellulose filter. The filter was hybridized at 42°C overnight in the hybridization solution with full-length OAT1 cDNA labeled with ^{32}P-dCTP. The filter was washed finally in 0.1x SSC/0.1% SDS at 65°C.

Under high stringency Northern blot analysis, a strong 2.4 kb mRNA band and two bands corresponding to longer transcripts (3.9 kb and 4.2 kb) were detected predominantly in the kidney (Fig. 4). In the kidney, expression of OAT1 mRNA is strong in the cortex and outer medulla (cortex > outer medulla) and very weak in the inner medulla.

Upon longer exposure, a faint 2.4 kb mRNA band was detected in the brain. No hybridization signals were obtained with mRNA isolated from other tissues.

(4) Intrarenal expression of OAT1 mRNA analyzed by in situ hybridization

The intrarenal expression of OAT1 was examined by *in situ* hybridization analysis. *In situ* hybridization was performed as described elsewhere (Kanai, Y. and Hediger, M.A. (1992) Nature 360, 467-471, 1992) with some modifications. Briefly, after perfusion fixation with 4% paraformaldehyde, rat kidney was excised and postfixed in 4% paraformaldehyde. Five μm cryostat sections of rat kidney were used *in situ* hybridization.

^{35}S-labeled sense and antisense cRNA were synthesized from the full-length OAT1 cDNA (in pBlueScript SK-) using T7 or T3 RNA polymerase after linearization of plasmid DNA with Spe I or Xho I, respectively. The cryosections were hybridized with the probe overnight in the hybridization solution, and washed to a final stringency of 0.1X SSC at 37°C for 30 min.

In situ hybridization of rat kidney coronal sections revealed that OAT1 mRNA is expressed in renal cortex and outer medulla, especially in the medullary rays of the cortex. Expression of OAT1 was not found in the inner medulla. This overall pattern of in situ hybridization suggests that OAT1 is most strongly expressed in the middle portion of the proximal tubule (S2).

EXAMPLE 2 : FUNCTIONAL CHARACTERIZATION OF ORGANIC ANION TRANSPORTER 1 (OAT1)

(1) The effect of the preincubation of glutarate on the transport activity of OAT1

The effect of the preincubation of glutarate was investigated in the uptake experiment using the oocytes expressed with OAT1.

The uptake experiment using PAH was performed as described in the methods of EXAMPLE 1-(2). Oocytes injected with rat OAT1 cRNA only, or both rat OAT1 and rNaDC-1 cRNA were incubated in the ND96 solution containing ^{14}C-PAH for 1 hour after preincubated them in the ND96 solution with and without 1 mM of glutarate.

Figure 5 shows the dependence of OAT1-mediated ^{14}C-PAH uptake on the intracellular dicarboxylate (glutarate) concentration. The rate of ^{14}C-PAH uptake by oocytes via OAT1 is increased by preincubation of the oocytes with 1 mM glutarate. When oocytes co-expressing rNaDC-1 and OAT1 are preincubated with glutarate,

hey showed a further increase in the rate of ^{14}C-PAH uptake. This *trans*-stimulative effect of glutarate indicates that OAT1 is an organic anion/ dicarboxylate exchanger. Control oocytes are those which were not injected with cRNA.

(2) The sodium dependency of the transport activity of OAT1

The effect of the extracellular sodium ion on the OAT1-mediated uptake of PAH was examined.

The uptake experiment using PAH was performed as described in the methods of EXAMPLE 2-(1).. In this experiment, choline 96 solution, in which 96 mM sodium chloride was replaced with equimolar of choline chloride, was also used in addition to ND96 solution.

As shown in Fig. 6, replacement of extracellular sodium with choline had no effect on the rate of ^{14}C-PAH uptake, indicating that OAT1 is a sodium independent transporter. Control oocytes were those which were not injected with cRNA.

(3) The kinetic experiment

Transport rate of different concentrations of PAH via OAT1 was measured to obtain the kinetic parameters of OAT1.

The uptake experiment using PAH was performed as described in the methods of EXAMPLE 2-(1). ^{14}C-PAH uptake was measured for 3 minutes. As shown in figure 7, OAT1-mediated PAH uptake followed Michaelis-Menten kinetics, and the estimated Km value was 14.3 ± 2.9 μM (mean ± s.e.m., N=3). This values is similar to that previously reported for the basolateral organic anion transport system (80μM) (Ullrich, K.J. and Rumrich, G. Am. J. Physiol. 254, F453-462, 1988).

(4) The substrate selectivity of OAT1 examined by inhibition study

The effect of various anionic drugs on the PAH uptake in the oocytes injected with rat OAT1 cRNA.

The uptake experiment using PAH was performed as described in the methods of EXAMPLE 2-(1). In this experiment, 2 μM of ^{14}C-PAH uptake in oocytes injected with rat OAT1 cRNA was measured in the ND96 solution with and without 2 mM of various non-labeled substances.

As shown in figure 8, cis-Inhibitory effect was observed for structurally unrelated drugs. Cephaloridine (a β-lactam antibiotic), nalidixic acid (an "old" quinolone), furosemide and ethacrynic acid (diuretics), indomethacin (a nonsteroidal anti-inflammatory drug), probenecid (an uricosuric drug) and valproic acid (an antiepileptic drug) potently inhibited (>85%) OAT1-mediated ^{14}C-PAH uptake. An antineoplastic drug, methotrexate, moderately inhibited ^{14}C-PAH uptake. Endogenous compounds, such as prostaglandin E2, cyclic-AMP, cyclic-GMP and uric acid also inhibited ^{14}C-PAH uptake.

(5) The substrate selectivity of OAT1 examined by uptake experiment using labeled anionic substances

Several radio labeled compounds were examined whether they are taken up into oocytes via OAT1.

The uptake experiment using PAH was performed as described in the methods of EXAMPLE 2-(1). In this experiment, radio labeled substances were used as substrates in stead of ^{14}C-PAH. Control oocytes were those which were not injected with cRNA.

As shown in Fig. 9, ^{3}H-methotrexate, ^{3}H-cAMP, ^{3}H-cGMP, ^{3}H-prostaglandin

E2, ^{14}C-urate and ^{14}C-α-ketoglutarate were revealed to be transported into the oocytes expressing OAT1. In contrast, any uptake of ^{14}C-TEA (tetraethylammonium: a representative organic cation) and ^{3}H-taurocholic acid were not detected (data not shown).

EXAMPLE 3 : CLONING OF THE HUMAN ORGANIC ANION TRANSPORTER

Using rat OAT1 cDNA obtained in EXAMPLE 1-(2), human cDNA library was screened. Human cDNA library was constructed from human kidney poly (A)+ RNA (Clontech).

Sequence of the isolated cDNA clone (human OAT1 cDNA) was determined according to the methods described in Example 1. The coding region of the human OAT1 cDNA and the deduce amino acid sequence was determined as well.

The sequence of human OAT1 in both nucleotide and amino acid level is shown in the SEQUENCE NO. 2.

The sequence homology between rat OAT1 and human OAT1 was approximately 85 % and 79 %, in amino acid level and nucleotide level, respectively.

INDUSTRIAL APPLICABILITY

The present invention, organic anion transporter 1 (OAT1) and the gene encoding OAT1, is considered to be useful to clarify the molecular mechanisms underlying the pharmacokinetics and toxicokinetics, such as the drug elimination and drug-drug interaction. In addition, the screening system to identify the nephrotoxic drugs and the way to protect kidney from such nephrotoxic substances will be developed, since many agents causing renal insufficiency, such as β-lactam antibiotics

and NSAIDs (non-steroidal anti inflammatory drugs), have been suggested to be transported by OAT1, and OAT1 seems to be responsible for the accumulation of these nephrotoxicants in the kidney.